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(54) Title: MUCOSAL DELIVERY OF POLYNUCLEOTIDES

(57) Abstract

A composition for delivery of a polynucleotide to mucosal, neural, or other cells, comprising a GN1-binding protein and a polynucleotide in association with the binding protein; and a method for modulating immunity comprising administering the composition to an animal and expressing the polynucleotide whereby the animal generates an immune response to the product of the polynucleotide; and a method for gene therapy comprising administering to an animal a GM1-binding protein and a functional polynucleotide and expressing the polynucleotide in the animal whereby the function of the polynucleotide confers on the animal a therapeutic effect.

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MUCOSAL DELIVERY OF POLYNUCLEOTIDES

RELATED APPLICATIONS

This application claims priority to provisional application No. 60/001527, filed July 26, 1996 under 35 U.S.C. § 119(e).

FIELD OF THE INVENTION

This invention relates to the delivery of functional polynucleotides to mucosal and neural tissues of vertebrate animals, and to other tissues or cells that display the GM1 receptor. The functional nucleotides thus delivered can be therapeutic genes or they can operate to modulate the immune responses of the animal. The modulation may either increase or decrease the magnitude of a subsequent immune response in the animal.

BACKGROUND OF THE INVENTION

Mucous membranes cover the exposed surfaces of the aerodigestive and urogenital tracts, the eye conjunctiva, the inner ear, and the ducts of all exocrine glands. Due to their distribution, mucous membranes encounter numerous foreign particles and invading microbes constantly. These membranes are capable of trapping and degrading most of the particles and microbes they encounter. In addition, mucosal tissues have a specialized immune system. The cells of the mucosal immune system comprise the mucosa-associated lymphoid tissues (MALT).

MALT cells regularly encounter a vast array of foreign substances. A full-scale immune response against every foreign substance thus encountered would render impossible such a simple and vital process as ingesting proteinaceous food, for example. Therefore, the mucosal immune system must have the ability to selectively ignore most foreign epitopes while it mounts a vigorous response to certain others.

The phenomenon of acquired toleration of certain foreign epitopes is known as "oral tolerance" because it was first perceived through a diminished sensitivity to certain otherwise immunogenic substances if ingested in large enough quantities. It is now known that this "oral tolerance" can be acquired, at least temporarily and locally, by sufficient exposure of MALT cells to some foreign substances.

Many mucosal cells are characterized by the relative abundance of ganglioside GM1 in their plasma membranes. GM1 is a sialoceramide whose lipophilic ceramide portion diffuses into and associates with the phospholipid bilayer of the plasma membrane, while the oligosaccharide-sialic acid moieties of GM1 protrude out of the membrane and are displayed on the cell surface. GM1 is not limited to mucosal cells alone. In fact, GM1 is present at some level in many different cell types of the body, but most abundantly in neuronal membranes and membranes of mucosal cells.

GM1 can act as a receptor in the plasma membrane for proteins that bind its exposed oligosaccharide-sialic acid portion. The best known GM1 binding protein is the toxin of *Vibrio cholerae*, the cholera toxin (CT). CT consists of two types of subunits. The A1 fragment of the A subunit has ADP-ribosylation activity while the B subunit has GM1-binding activity. The holotoxin is a heterohexamer consisting of five B subunits and one A subunit. The structure and function of the related *E. coli* heat-labile enterotoxin (LT)is quite similar to that of CT.

The ADP-ribosylation activity and the GM1 binding of CT are both essential to the overall toxin activity of CT. Subunits of the holotoxin, whether A or B, do not separately cause the toxin reaction *in vivo*. In the cell, the A subunit's ADP-ribosylation activity transfers the ADP-ribosyl moiety of NAD to the α subunit of the guanine-nucleotide-binding protein G_s, the membrane-bound positive regulator of adenylate cyclase.

Although the present text primarily discusses the use of GM1 binding proteins, it should be noted that "ADP-ribosylating moieties" are also contemplated as part of the present invention as fully as if that term was used in place of the term "GM1 binding protein," as the latter term appears herein.

There are various other ADP-ribosylating enzymes with analogous function. For example, the heat-labile enterotoxin (LT) of *E. coli* is doubly similar to CT because its A and B subunits also display ADP-ribosylation activity and GM1 binding activity, respectively.

Because GM1 includes and displays an oligosaccharide portion, it can also be bound by numerous lectins and lectin-like molecules. Also, because of the lipophilic nature of the ceramide portion, exogenous GM1 is readily taken up by membranes of many cell types.

CT elicits a strong reaction by the mucosal immune system. Accordingly, the holotoxin or the A subunit alone have been used as adjuvants to stimulate a mucosal immune reaction against other selected epitopes. Surprising to many is the contrasting fact that CTB, when coupled to a selected epitope, has a strong ability to effect an acquired ("oral") tolerance to the delivered epitope. The B subunit alone efficiently delivers the coupled epitope to the proximity of the MALT, but generally does not by itself stimulate an enhancement of immune recognition. The result of such a delivery without a stimulus of immunity is often acquired tolerance.

The dual phenomena of enhanced or diminished mucosal immunity, together with the influence that the mucosal immune system has on systemic immunity, have caused considerable interest in the development of compositions and methods of treatment involving CT or CTA as adjuvants for mucosal immunity, and CTB as an initiator and mediator of acquired tolerance.

Current methods and compositions rely on covalent coupling of a desired epitope to CTB for delivery of the epitope to the MALT with the purpose of gaining acquired tolerance to the epitope. This scheme has been especially appreciated by those interested in addressing problems of allergy, allograft rejection, and autoimmune diseases.

Likewise, workers wishing to stimulate acquired mucosal, and possibly systemic, immunity have combined their selected antigen with CT or CTA to achieve enhanced mucosal immunity.

In both cases, the instability of the delivered protein epitope and of the coupling to CTB have been limitations to the effectiveness and duration of the desired exposure of MALT cells to the epitope. The invention described herein addresses this problem in a novel way. Beyond the novelty of the solution to a shortcoming in the current state of the art, the present invention also has great utility in gene therapy applications.

Described herein is an invention that delivers an active polynucleotide to target cells, such as MALT cells, by complexing or coupling the polynucleotide to a GM1- or other mucosa-binding protein. The delivered molecule, being a polynucleotide rather than a protein, has an effect in a single delivery event that can be many hundred- or thousand-fold greater than the delivery of a single protein. This is because the active polynucleotide can direct,

encode, or otherwise affect the ultimate expression of many copies of the desired protein. Further, it can do so for hours or days if expressed transiently, or on a permanent basis if the polynucleotide stably integrates into the genome of the cell to which it is delivered. Similar results can not possibly be achieved when the delivered molecule is merely a protein.

Since the invention employs binding proteins that do not only recognize mucosal cells, the invention also has utility in the field of gene therapy. The binding protein and membrane determinants of the target cell can be selected and/or manipulated such that a therapeutic gene or other polynucleotide can be faithfully and efficiently delivered to the target cell. The result is either a transient transfection or a stable, permanent transformation or the target cell.

SUMMARY OF THE INVENTION

This invention introduces a novel approach to gene therapy and to manipulation of the immune system. The invention relates to a composition that possesses the ability to localize functional polynucleotides to mucosal tissues, neural tissues, and to other tissues or cells that display the GM1 receptor. The functional nucleotides thus delivered can be therapeutic genes or they can operate to modulate the immune responses of the animal. The modulation may either increase or decrease the magnitude of a subsequent immune response in the animal.

The invention is embodied in a composition for delivery of a polynucleotide to mucosal cells, nervous cells, and other vertebrate cells and tissues. The composition comprises a mucosa-binding protein having an affinity for determinants of vertebrate mucosal tissues in association with an active polynucleotide. Because the mucosa-binding protein also has an affinity for other cell types, the composition of the invention is also effective in delivering an active polynucleotide to other selected cells and tissues as well.

A more particular embodiment of the invention has as the protein component a member of the following group of mucosa-binding proteins: CT, CTB, LT, bacterial fimbriae proteins, animal lectins, plant lectins, and lectin-like proteins.

In a preferred embodiment of the invention, the mucosa-binding protein is the cholera toxin of *Vibrio cholerae*. This embodiment contemplates the use of the holotoxin, which consists of A and B subunits, or monomers or fragments thereof, and also contemplates the use of a particular fragment of the toxin, consisting only of the B (binding) subunit. Also contemplated is the use of only the A (active) subunit, involving both fragments of the A subunit (A1 and A2) or only one fragment thereof, as well as other molecules displaying ADP-ribosylation activity.

Another embodiment of the invention has as its protein component an immunoglobulin capable of specifically recognizing mucosal cells. In a more particular embodiment of this aspect of the invention, the immunoglobulin recognizes and binds to the GM1 receptor.

Further embodiments of the invention relate to the polynucleotide component. In one embodiment, the polynucleotide is DNA. Where the polynucleotide is DNA, the DNA may be of genomic origin. Alternatively, the DNA may be cDNA. Additionally, according to the invention, the DNA may be recombinant DNA. The invention contemplates all forms of DNA as the polynucleotide component.

This aspect of the invention has as a preferred embodiment DNA that contains a promoter region operationally linked to a coding region. The coding region may specify various protein domains. Another preferred embodiment of the invention has DNA that encodes an antisense message.

In another embodiment of the invention, the polynucleotide component is RNA. Accordingly, in a preferred embodiment, the RNA is mRNA. In another preferred embodiment, the RNA is antisense. In an additional embodiment, the RNA is a ribozyme. The invention contemplates all forms of RNA as the polynucleotide component.

The composition may exist in several useful forms. All useful forms of the composition are contemplated in this invention. In one embodiment, the composition exists in the form of an atomized dispersion, such as would be used in delivery by inhalation. In a preferred embodiment, the composition exists in the form of a suppository, whether rectal or vaginal. In another preferred embodiment, the composition exists in the form of a cream, an ointment, or a transdermal patch.

The invention also contemplates all ways in which the mucosa-binding protein and the polynucleotide may form the composition. In a preferred embodiment, the composition exists in the form of a mixture of mucosa-binding protein and polynucleotide. The mixture may be stabilized by other components that exploit electrostatic or hydrophobic interactions, or that diminish the effectiveness of mucus as a barrier to delivery of the polynucleotide. In another embodiment, the mucosa-binding protein and the polynucleotide are chemically coupled to each other. In an additional embodiment of the invention, the mucosa-binding protein and the polynucleotide are encapsulated together.

Another aspect of the invention is a method for modulating immunity in an animal. The first step of the method is to administer to an animal in combination a mucosa-binding protein and a polynucleotide encoding an immunogen. The second step is to express the polynucleotide in the animal whereby the animal generates an immune response to the product of the polynucleotide.

In one preferred embodiment of this aspect of the invention, the modulation is an enhanced immune response in the animal. The invention contemplates that the enhanced immune response may be mucosal, or systemic, or both mucosal and systemic. An additional embodiment of this aspect of the invention directs the enhanced immune response to contraception.

In another preferred embodiment of the invention, the modulation is a diminished immune response. The invention contemplates that the diminished immune response may be mucosal, or systemic, or both mucosal and systemic. The invention is preferably embodied in a diminished immune response for desensitization to allergens, in a manner analogous to oral tolerance. In another preferred embodiment, the diminished immune response is for toleration of organ and tissue transplants. A further preferred embodiment of the invention directs the diminished immune response to the treatment of an autoimmune disease.

According to the invention, the modulation of the immune response may be done prior to exposure to a pathogen or allergen. The invention also contemplates the modulation where it is done after exposure to the pathogen or allergen. Therefore, all uses of the composition of the invention for the modulation of the immune

response are embodied in the present invention, whether preexposure or postexposure, and whether to immunize or desensitize the animal so treated.

The composition of the invention may be administered in a number of ways. In one embodiment, the mode of administration is nasal and/or pulmonary. In a preferred embodiment, the mode of administration is oral. Further preferred embodiments administer the composition of the invention rectally or vaginally. Alternatively, the invention is also embodied in a mode of administering the composition of the invention by inhalation, by administering the composition ophthalmically, and also by administering the composition transdermally.

Another aspect of the invention is the composition functioning to deliver a polynucleotide to any GM1 animal cell. The binding protein component of the composition has an affinity for the GM1 receptor and because of the affinity, delivers a polynucleotide to any cell displaying the GM1 receptor.

In one embodiment of this aspect of the invention, the binding protein is selected from the group consisting of CT, CTB, LT, LTB, bacterial fimbriae proteins, plant lectins, animal lectins, and lectin-like proteins.

In a preferred embodiment, the binding protein is either cholera holotoxin (with A and B subunits) or it is a derivative of the toxin, having some fragment of the toxin, either alone or in combination with other protein fragments.

In an alternative preferred embodiment, the binding protein of the composition is an immunoglobulin.

According to the invention, GM1 delivery of a polynucleotide also contemplates purposes of gene therapy. As such an embodiment, the invention discloses a method for gene therapy in an animal by administering to an animal in combination a GM1-binding protein and a functional polynucleotide and then expressing the polynucleotide in cells of the animal. By this method, the function of the polynucleotide confers on the animal a therapeutic effect.

In a preferred embodiment of this aspect of the invention, the cells of the animal transiently express the delivered functional polynucleotide.

An alternative preferred embodiment exists when the cells of the animal are stably transformed by the functional polynucleotide.

DETAILED DESCRIPTION OF THE INVENTION

Mucosal tissues are the route of entry of many infectious diseases. They are also the chief sites of invasion and irritation by many allergens. This invention addresses both of these medical problems. Furthermore, the invention provides an approach to other significant diseases and conditions via a novel method of delivering polynucleotides to certain cells for gene therapy.

The invention makes possible the modulation of the mucosal and systemic immune systems of vertebrate animals by targeting functional polynucleotides to the cells involved in mucosal immunity. The polynucleotides are then internalized within these cells and are expressed therein. The products of the polynucleotides can either enhance or diminish the magnitude of the body's normal immune response to the epitope of choice, thus rendering the body less reactive to certain allergens, or more effective at combating certain pathogens, according to the parameters of

the particular use of the invention. The ability to thus modulate the responses of the immune system also relates the invention to the treatment and prevention of autoimmune diseases.

Rather than delivering only one dose of a particular polypeptide allergen or immunogen, as would be the case with a composition consisting of a mucosa-binding protein and a given polypeptide, the present invention makes possible a greater exposure of the target cells to a given allergen or immunogen because of the fact that a single polynucleotide can direct the expression of many copies of its product. The effectiveness of the exposure of the mucosal cells to the peptide product may also be improved because of the duration of the expression of the polynucleotide within the cell, and because of the intracellular origin of the polypeptide product of the active polynucleotide.

Because the mucosa-binding proteins of the invention will actually bind to any cells that display GM1 in sufficient concentrations, the invention further constitutes a novel mode of polynucleotide targeting and delivery for gene therapy applications. Current modes of therapeutic gene delivery are largely limited to viral capsids, cationic lipids, engineered live viruses, and *ex vivo* transfection. Most require injection of the material in question. However, this invention makes possible the administration of a therapeutic gene or other functional polynucleotide by means of inhalation, oral dosage, eye drops or creams, nose drops or creams, suppositories, enemas, douches, or transdermal patches, creams, or ointments. Accordingly, the use of the present invention for gene therapy will have special effectiveness against diseases of mucosal tissues and neural tissues, and against other diseases amenable to treatment by polynucleotide delivery to tissues the cells of which display GM1 receptors.

Specifically, the present invention discloses a composition for delivery of functional polynucleotides to various cells of vertebrates. The invention comprises a binding protein and a functional polynucleotide. The protein component of the composition has the property of recognizing and binding to determinants of many vertebrate cells. It may further have the property of protecting itself and other molecules with which it is associated from rapid degradation within the body of the animal. And, being associated with a functional polynucleotide, the binding protein has the important property of delivering the polynucleotide to target cells.

The present invention contemplates the use of any protein capable of specifically binding to or associating with any of the several neural or mucosal tissues, including dendritic tissues, tissues of the gastrointestinal tract, the reproductive tract, pulmonary airways including lungs, nasal sinuses, and the conjunctiva of the eyes. As nonlimiting examples, the following proteins are all known to bind mucosal tissues: bacterial toxin membrane binding subunits including, at a minimum, the B subunit of cholera toxin, the B subunit of the *E. coli* heat-labile enterotoxin, *Bordetella pertussis* toxin subunits S2, S3, S4 and/or S5, the B fragment of Diphtheria toxin and the membrane binding subunits of Shiga toxin or Shiga-like toxins.

Other mucosa-binding subunits contemplated within the scope of this invention include the bacterial fimbriae proteins *E. coli* fimbria K88, K99, 987P, F41, CFA/I, CFA/II (CS1, CS2 and/or CS3), CFA/IV (CS4, CS5 and/or CS6), P fimbriae, or the like. Other fimbriae contemplated within the scope of this invention include *Bordetella pertussis* filamentous hemagglutinin, *Vibrio cholerae* toxin-coregulate pilus (TCP), mannose-sensitive hemagglutinin (MSHA), fucose-sensitive hemagglutinin (FSHA), and the like.

Still other mucosa-binding molecules contemplated within the scope of this invention include viral attachment proteins including influenza and Sendai virus hemagglutinins and animal lectins or lectin-like molecules including immunoglobulin molecules or fragments thereof, calcium-dependant (C-type) lectins, selectins, collectins or *Helix pomatia* hemagglutinin. Plant lectins with mucosa-binding subunits include concanavalin A, wheat-germ agglutinin, phytohemagglutinin, abrin and ricin.

Other proteins that can specifically recognize and bind to neural, mucosal, or other vertebrate cells are contemplated in this invention. Specifically, any immunoglobulin having an affinity for distinctive mucosal epitopes, such as the GM1 receptor, is part of the mucosa-binding protein component contemplated in the present invention. Additionally, recombinant, chimeric, or otherwise engineered proteins possessing the property of binding to mucosal cells, are also contemplated as part of this invention.

In a preferred embodiment of the invention, the mucosa-binding protein is the cholera toxin of *Vibrio cholerae*. This embodiment contemplates the use of the holotoxin, which consists of A and B subunits, and also contemplates the use of a fragment of the toxin, consisting only of the B (binding) subunit monomers either singly or in multimers.

The invention is further embodied in the use of the A (active) subunit of CT or its analogs as the protein component of the composition. This aspect of the invention contemplates the use of only the A1 fragment, or only the A2 fragment, as the protein component of the present invention. Additionally, other enzymes, hormones, cofactors, or catalysts that, like A1, possess ADP-ribosylation activity or aid therein, also embody this aspect of the invention. The invention further contemplates use of derivatives of the cholera toxin, whether natural or synthetic, including all or part of either subunit.

Another embodiment of the invention has as its protein component an immunoglobulin capable of specifically recognizing mucosal cells. In a more particular embodiment of this aspect of the invention, the immunoglobulin recognizes and binds to the GM1 receptor. Immunoglobulins raised against other determinants of mucosal cells are also contemplated in this invention.

The binding and toxin properties of the cholera toxin are well known in the art. Many other proteins with analogous binding properties are also known. Still others can be engineered. Likewise, immunoglobulins against GM1 or other specific epitopes of mucosal cells can be raised.

Further embodiments of the invention relate to the polynucleotide component. In one embodiment, the polynucleotide is DNA. Where the polynucleotide is DNA, the DNA may be of genomic origin. Genomic DNA can be DNA with or without introns, and with or without promoter sequences, enhancer sequences, and other cis-acting elements. Genomic DNA may also include DNA of genomic origin which has subsequently been manipulated by recombinant DNA techniques. Genomic DNA may be originally derived from animals, plants, fungi, protists, or bacteria.

Alternatively, the DNA may be cDNA. For purposes of this invention, cDNA may be directly made from mRNA via reverse transcriptase, or it may be cDNA-like molecules, where the DNA is not made from mRNA with

reverse transcriptase, but rather is manipulated to remove introns and to add or remove other sequences such that the product of the manipulations has the general characteristics of cDNA.

Additionally, according to the invention, the DNA may be recombinant DNA. For purposes of this invention, recombinant DNA may be any DNA that has been subject to manipulation by recombinant DNA techniques. Such techniques may include, but are not limited to: random mutagenesis, site-directed mutagenesis, 3' or 5' exonuclease trimming, linker addition, ligation with other DNA sequences, methylation, demethylation, polyadenylation, insertion of a foreign fragment, and removal of an endogenous fragment.

The DNA may also be random fragments of DNA. For random fragmentation, the initial DNA may be a selected amplified sample of DNA, or it may be a random sample of genomic DNA.

Accordingly, the polynucleotide component of the invention is not limited only to sequences that encode protein products. Any functional DNA sequence is contemplated, without reference to the length of the molecule in base pairs, and without reference to the physical structure of the molecule. Therefore, whether the DNA is in plasmid form, either supercoiled or open circular, concatameric, linear, single-stranded, or double-stranded, the invention contemplates any functional DNA sequence as the polynucleotide component.

This aspect of the invention has as a preferred embodiment DNA that contains a promoter region and a coding region. Where there is a promoter region, promoters of any origin are contemplated.

Coding regions may specify a particular polypeptide. Such coding regions may include introns or may be without introns. In addition, other kinds of coding regions may be used. Nonlimiting examples of other kinds of coding regions are sequences that encode an antisense message or sequences that constitute competitive targets for DNA binding proteins or other agents of intermolecular interactions.

When the coding region does specify a polypeptide, the invention contemplates all forms of coding regions, both naturally occurring and recombinant. The sequences may be derived from random or site-directed mutagenesis, or they may be non-mutant sequences. They may also specify various protein structures and domains, such as helices, sheets, signal peptides, transit peptides, targeting domains, retention domains, hydrophobic domains, hydrophilic domains, fingers, zippers, loops, coils, active sites, binding sites, processing sites, cleavage sites, oligomerization moieties, and the like. They may also specify random fragments of known or predicted protein sequences.

This aspect of the invention is further embodied in DNA derived from a virus. Such DNA may be directly purified from a DNA virus or may be purified from a viral host, whether eukaryotic or prokaryotic. In addition, the viral DNA may be synthesized *in vitro*. For purposes of this invention, viral DNA includes any DNA molecule of a virus or derived from a viral RNA or DNA sequence, whether the sequence is complete or only a portion or fragment of the sequence is represented.

Another preferred embodiment of the invention has DNA that encodes an antisense message. For purposes of this invention, an antisense message is any polynucleotide sequence capable of complementarily associating with other polynucleotide sequences that would otherwise be translated, or would assist in translation. This definition encompasses polynucleotides that can form an antisense hybrid with, for example, MRNA, tRNA, rRNA, or other RNA

molecules, regions of RNA molecules, or other active polynucleotides. The essential nature of such antisense messages is that they are sequences capable of binding to other polynucleotides and either eliminating or attenuating their translation or other expression or activity. DNA encoding an antisense RNA is also within the scope of this invention.

Techniques of DNA selection and manipulation required to practice this invention are well known, such that the kinds of DNA polynucleotides discussed herein could be prepared and used by one skilled in the art without undue experimentation.

In another embodiment of the invention, the polynucleotide component is RNA. All functional forms of RNA are contemplated in the invention, regardless of origin, structure, modifications, or method of purification.

In a preferred embodiment, the RNA is MRNA. For purposes of this invention, MRNA is any RNA translated or capable of being translated to create a polypeptide product. Therefore, MRNA can be purified from cells, either eukaryotic or prokaryotic, or it can be synthesized *in vitro*. The invention contemplates MRNA molecules with and without modifications affecting efficiency of translation, such as, for example, polyadenylation at the 3' end or a 5' m^7G cap. In another preferred embodiment, the RNA is antisense RNA. For purposes of this invention, antisense RNA is any RNA sequence capable of complementarily binding with other polynucleotide sequences that would otherwise be translated, or would assist in translation. This definition encompasses polynucleotides that can form an antisense hybrid with, for example, mRNA, tRNA, rRNA, or other RNA molecules, regions of RNA molecules, or other active polynucleotides.

The concept of antisense interference with or attenuation of polynucleotide activity is well known in the art, and a person of ordinary skill would be able to design, isolate, or engineer an antisense polynucleotide likely to have the desired effects.

This aspect of the invention is further embodied in RNA derived from a virus. Such RNA may be directly purified from an RNA virus or may be purified from a viral host, whether eukaryotic or prokaryotic. In addition, the viral RNA may be synthesized *in vitro*. For purposes of this invention, viral RNA includes any RNA molecule of a virus or derived from a viral RNA or DNA sequence, whether the sequence is complete or only a portion or fragment of the sequence is represented.

In an additional embodiment, the RNA is a ribozyme. For purposes of this invention, a ribozyme is any RNA molecule with a catalytic activity, whether autocatalytic or heterocatalytic. The use of any ribozyme as the polynucleotide component of the composition of the invention is contemplated herein.

Other forms and conformations of RNA are also part of this invention. As nonlimiting examples, the RNA may be rRNA, tRNA, hnRNA, single-stranded, double-stranded, self-annealed, looped, or concatameric.

Additionally, the polynucleotide component of the invention is embodied in any functional polynucleotides that are not listed above, whether existing in nature or synthetic. Non-limiting examples of such polynucleotides contemplated by the invention are: linkers, genes, promoters, introns, enhancer sequences, silencer sequences, transposable elements, RNA/DNA hybrids, RNA/DNA chimeras, DNA sequences containing nucleotide analogs, RNA

containing nucleotide analogs, heterogeneous mixtures of different DNA sequences, heterogeneous mixtures of different RNA sequences, and heterogeneous mixtures of both DNA and RNA sequences.

The composition may exist in several useful forms. All useful forms of the composition are contemplated in this invention. In one embodiment, the composition exists in the form of an atomized dispersion for use in delivery by inhalation. The atomized dispersion may be a solution of the composition of the invention, or it may be a suspension containing the composition. Typical carriers for atomized or aerosolized dispersions include Miglyol®, Frigen® (11/12/113/114), and buffered saline. The delivery of the composition of the invention via inhalation has the effect of rapid dispersion to a large area of mucosal tissues, as well as absorption by the blood for circulation of the composition of the invention to other GM1 cells of the body.

In a preferred embodiment, the composition exists in the form of a suppository, whether rectal or vaginal. Typical carriers for formulation of the inactive portion of a suppository include polyethylene glycol, glycerine, cocoa butter, Imhausen H, and Witepsol® H. Other suppository formulations suitable for delivery of the composition of the invention are also contemplated as part of the invention. Delivery of the composition of the invention via suppository has the effect of contacting a mucosal surface with the composition for release to proximal mucosal tissues. Distal mucosal tissues may also receive the composition of the invention by diffusion.

Additionally, the invention contemplates the composition existing in the form of a liquid. The liquid may be for oral dosage, or for ophthalmic or nasal dosage as drops or for use as an enema or douche. When the composition of the invention exists in the form of a liquid, the liquid may be either a solution or a suspension of the composition. There is a variety of suitable formulations for the solution or suspension, depending on the intended use thereof.

Delivery of the composition of the invention in liquid form via oral dosage has the aim of exposing the mucosa of the gastrointestinal tract to the composition. A suitable dose, stabilized to resist the pH extremes of the stomach, would deliver the composition to all parts of the gastrointestinal tract, especially the upper portions thereof. The invention contemplates all means of stabilizing the composition in a liquid oral dosage such that the effective delivery of the composition could be evenly distributed along the gastrointestinal tract. For example, a coated or controlled release material can be used to release the composition only after it traverses the stomach. For oral administration in liquid form, the composition could be thus coated in fine particles and be administered as a suspension.

Delivery of the composition of the invention in liquid form via ophthalmic drops would be with the aim of exposing the mucosa of the eyes and associated tissues to the composition. A typical liquid carrier for eye drops is buffered saline containing Methocel® 65 HG 4000.

Delivery of the composition of the invention in liquid form via nasal drops would be with the aim of exposing the mucosa of the nose and sinuses and associated tissues to the composition. Liquid carriers for nasal drops are typically various forms of buffered saline.

The invention is further embodied in the composition in the form of a cream for ophthalmic, nasal, vaginal, or rectal administration. The use of the composition of the invention in the form of a cream applied to a given

mucosal tissue has the aim of contacting the composition with the mucosal tissue for a sufficient time to allow the effective delivery of the composition to the tissue. The cream may also have properties of solubilizing mucus or otherwise diminishing the effect of mucus as a barrier to delivery of the composition. Additional mucolytic agents such as DNase solutions may also be added. Typical creams and ointments use as carriers paraffin oil, petrolatum, glycerol, polyethylene glycol, propylene glycol and Methocel®.

The invention is also embodied in forms of the composition appropriate for topical application or transdermal delivery to dendritic and other cells. Contemplated forms include transdermal patches, creams, and ointments. Transdermal delivery has the aim of releasing the composition either rapidly or gradually over time, to cells proximal to dermal tissues. Such cells include dendrites and other nervous tissue, blood cells, and smooth and striated muscle cells.

In another embodiment of the invention, the composition exists as a solid tablet or in a capsule form, for oral dosage. The use of the composition in this form has the effect of delivering the composition to the gastrointestinal tract for exposure of the intestinal mucosa to the composition. Accordingly, the tablet or capsule may consist of a material effective in protecting the composition from pH extremes of the stomach, or effective in releasing the composition over time, to optimize the delivery of the composition to the intestinal mucosa. The enteric coating for acid-resistant tablets and capsules typically includes cellulose acetate phtalate, propylene glycol, and sorbitan monoleate.

The invention also contemplates all ways in which the mucosa-binding protein and the polynucleotide may form the composition. In a preferred embodiment, the polynucleotide and the protein are mixed, without the intentional formation of covalent bonds between the polynucleotide and the protein. In this embodiment, other ingredients may be included in the mixture to enhance and stabilize the association between the protein and the polynucleotide. The proper mixing ratios of the protein component and the polynucleotide component can easily be empirically determined depending on the desired effects of the mixture.

The present ways of stably but noncovalently joining a binding protein and a polynucleotide together include the polycationic polylysine method and the cationic liposome method. In the polylysine method the binding protein is first chemically coupled to polylysine. The polycationic polylysine coupled with the binding protein is then mixed with the polynucleotide. The electrostatic interactions between the polylysine and the nucleic acid create a stable composition that does not easily dissociate and is therefore suitable for delivery to the target cells. Wu, G.Y., Wilson, J.M., Shalaby, F., Grossman, M., Shafritz, D.A., and Wu, C.H. (1991) J. Biol. Chem. 266:14338-14342; Chen, J., Gamou, S., Takayanagi, A., Shimizu, N. (1994) FEBS Lett. 338:167-169; both incorporated herein by reference.

Another nonlimiting example of the technology is the use of positively charged lipids to form liposomes capable of electrostatically interacting with polynucleotides. The polynucleotide is mixed with the lipids and liposomes are formed. A bifunctional component of the liposome is then chemically coupled to a derivatized form of the binding protein of the invention. The composition is thus capable of delivering the polynucleotide to the target cells without the formation of any covalent bonds between the polynucleotide and the protein. Mizuno, M., Yoshida,

J., Sugita, K., Inoue, I., Seo, H., Hayashi, Y., Koshizaka, T., and Yagi, K. (1990) Cancer Res. 50:7826-7829; incorporated herein by reference.

In another embodiment, the mucosa-binding protein and the polynucleotide are chemically coupled to each other. Various linkages are contemplated. As nonlimiting examples, the linkage may be either at the carboxy terminus of the protein, the amino terminus of the protein, or at an R group of an amino acid residue within the protein. The polynucleotide may be linked at the 5' end, the 3' end, or with some polynucleotide conformations, the linkage may be internal to the polynucleotide sequence.

In another preferred embodiment of the invention, the mucosa-binding protein and the polynucleotide are encapsulated together. The encapsulation means may play the dual role of maintaining the association between the components of the composition while also protecting the composition from stomach acid, digestive enzymes, excessive mucus, or other obstacles to optimal delivery to the preferred mucosal tissue. The great variety of possible encapsulating means is well known in the art. The invention contemplates the use of any encapsulating means suitable for a particular application.

Another aspect of the invention is a method for modulating immunity in an animal. For purposes of this invention, modulating immunity includes either enhancing or diminishing an immune response, or it may also include effecting both enhancement and diminution simultaneously, as discussed below. The first step of the method is to administer to an animal in combination a mucosa-binding protein and a polynucleotide encoding an immunogen. The second step is to express the polynucleotide in the animal, whereby the animal generates an immune response to the product of the polynucleotide.

In one preferred embodiment of this aspect of the invention, the modulation is an enhanced immune response in the animal. Modulation for enhancement may be characterized by a more rapid immune response than would otherwise occur. It may also be characterized by a higher titer of immunoglobulin after exposure to the antigen. Additional characteristics may include an increased resistance to a pathogen, a shorter duration of infection by a pathogen than would otherwise occur, an increased reactivity to related pathogens or antigens, or increased inflammatory response to the antigen. The invention contemplates that the enhanced immune response may be mucosal or systemic, or both mucosal and systemic. Mucosa-binding proteins such as CT are known to be effective in delivering polypeptides for generating an immune response. Use of these proteins to deliver polynucleotides encoding antigens is similarly effective.

In addition to the indicators of enhanced immune responses listed above, a mucosal enhanced immune response may be characterized by an increased production of neutralizing antibodies including sIgA. It may be further characterized by increased production of immunoglobulins IgE, IgG and by enhanced cell-mediated immunity.

In addition to the indicators of enhanced immune response listed above, a systemic enhanced immune response may be characterized by an increased production of neutralizing antibodies including immunoglobulin IgG. It may be further characterized by increased production of immunoglobulin IgA and by enhanced cell-mediated immunity.

An additional embodiment of this aspect of the invention directs the enhanced immune response to contraception. An acquired specific mucosal immunity to determinants unique to sperm cells is contemplated. Where such an immunity is generated, the animal so immunized would react to clear mucosal tissues of sperm cells, thereby decreasing the number of active sperm available to fertilize an egg of the animal.

In another preferred embodiment of the invention, the modulation is a diminished immune response. Modulation for a diminished immune response may be characterized by a less rapid immune response than would otherwise occur. It may also include a lower titer of immunoglobulin after exposure to the antigen. Additional characteristics may include an increased tolerance of an allergen or related pathogens or antigens, diminished or eliminated allergy symptoms, or down-regulation of inflammatory cytokines. The invention contemplates that the diminished immune response may be mucosal, or systemic, or both mucosal and systemic.

The invention is preferably embodied in a diminished immune response for desensitization to allergens. The invention contemplates varying degrees of desensitization.

One example of desensitization to allergens is the well known phenomenon of oral tolerance. In classic oral tolerance, an animal that is exposed to relatively large quantities of a particular substance, for example, an allergen, over time becomes less immunologically sensitive to the allergen. The oral tolerance thus developed can manifest itself both in the mucosal immune system and in the systemic immune system.

The dosage required to develop oral tolerance can be significantly reduced using mucosa-binding proteins coupled to the allergen. In experiments delivering human gamma globulin (HGG) to mice either via simple oral dosage (feeding) or with a mucosa-binding protein (CTB) linked to the antigens, the amount of antigen required to achieve the same level of tolerance to delayed-type hypersensitivity (DTH) (late phase) was approximately 200- to 625-fold greater with simple feeding than with coupling to CTB. Furthermore, the delivery by simple feeding was ineffective in diminishing the early phase of the DTH response, while the delivery of relatively small doses via coupling to a mucosa-binding protein was fully effective. Sun, J.-B., Holmgren, J., and Czerkinsky, C. (1994) Proc. Nat. Acad. Sci. USA 91:10795-10799; incorporated herein by reference.

The present invention likewise exploits the capacity of the mucosal immune system to attenuate the body's immune response to certain allergens or other immunogens, but not by delivery of protein allergens or immunogens themselves. Rather, this invention delivers active polynucleotides to the mucosa in order that the products or the activities of the polynucleotides may play a role in the diminution of the body's immune response. It is important to note that the polynucleotides themselves are not the immunogens, antigens, allergens, or haptens, but instead it is the polypeptide products, or the other activities of the polynucleotides, that ultimately effect the desired attenuation of the immune response.

Because the particular protein component of the composition has a great effect on whether the immune response is modulated toward enhancement or diminution, the invention contemplates compositions capable of either kind of modulation, as well as modulation of either type of immunity, whether mucosal or systemic.

In another preferred embodiment, the diminished immune response is for toleration of organ and tissue transplants. Exogenous nonself signals of the MHC I class of proteins are capable of stimulating an immune

response, and their genes are therefore candidates for use in the composition of the invention in order to eliminate or at least attenuate the immune response associated with recognized nonself epitopes on transplanted organs and tissues.

As a nonlimiting example, delivery of a gene encoding an MHC I protein to the mucosa via combination with a mucosa-binding protein results in local expression of the gene in mucosal cells. The gene product, containing nonself epitopes, would normally stimulate a significant immune response. Instead, because of the nature of its expression in mucosal cells, it effects a diminished sensitivity to the epitopes, which diminished sensitivity is manifest in both the systemic and mucosal immune systems. Shortly thereafter, a transplant that displays the same epitopes is placed in the animal, and the immune response directed against the transplanted tissue is much weaker than it would otherwise have been. Accordingly, the chances of rejection are decreased, as is the need for administration of immune-suppression drugs to the transplant recipient.

A further preferred embodiment of the invention directs the diminished immune response to the treatment of an autoimmune disease. Exogenous nonself epitopes are not the only determinants recognized and attacked by the immune system. Some abnormalities of the immune system result in a misrecognition of self as nonself, accompanied by a mistaken immune reaction against the animal's own cells and tissues. This phenomenon is the basis for autoimmune diseases such as, for example, systemic lupus erythematosis, type I diabetes, rheumatoid arthritis, and multiple sclerosis. Where such a condition exists, desensitization of the immune system to the misrecognized determinants of self cells eliminates or ameliorates the condition. Accordingly, the invention contemplates the use of the composition of the invention to treat or cure autoimmune diseases.

Also contemplated in the present invention is the immunization against certain epitopes of a disease pathogen together with a simultaneous desensitization to other epitopes of the pathogen. The effect of such a simultaneous up- and down- regulation of the immune system is to focus the effective immune defenses on the pathogen so as to eventually control or eliminate it, while reducing "bystander tissue" damage due to unproductive collateral inflammation. In cases where different epitopes stimulate the cell-mediated immune response on one hand and the injurious inflammatory response on the other, the disease can best be treated by simultaneous modulation of the immune response toward greater sensitivity to one epitope and much less responsiveness to the other.

According to the invention, the modulation of the immune response may be prior to exposure to a pathogen or allergen. Nonlimiting examples of application of the invention using preexposure modulation include vaccinations, pretransplant desensitization, and preemptive autoimmune deterrence.

Where a pathogen is known, and vaccination against the pathogen is desired, a gene encoding an epitope of the pathogen is delivered as part of the composition of the invention. The gene is internalized and expressed within mucosal cells, and an enhanced immunity of both the mucosal and the systemic immune systems results. Subsequent exposure to the pathogen is met at the point of contact by a strong and specific mucosal immunity as well as systemic immunity. The effectiveness of the pathogen as a disease causing agent is thus reduced or diminished.

The invention also contemplates the modulation where it occurs after exposure to the pathogen or allergen. Nonlimiting examples of applications of the invention using postexposure modulation include postexposure immunization

for chronic infections, immunization to create an autoimmune reaction to cancer cells, desensitization treatment for allergies, and therapeutic desensitization to epitopes attacked in autoimmune disease.

Where an autoimmune disease has been diagnosed and its basis is understood, a polynucleotide encoding the epitope being misrecognized as nonself by the immune system is complexed or otherwise combined with CTB. The composition is fed to the animal in an appropriate dose, which results in diminished or eliminated mucosal and systemic immunity against the epitope of the autoimmune disease.

Accordingly, all uses of the composition of the invention for the modulation of the immune response are embodied in the present invention, whether preexposure or postexposure, and whether to immunize or desensitize the animal so treated.

The composition of the invention may be administered in a number of ways. In one embodiment, the mode of administration is nasal. Administration of the composition of the invention nasally has the aim of exposing the mucosa of the nose and sinuses and associated tissues to the composition. Alternatively, the invention is also embodied in a mode of administering the composition of the invention by inhalation. Administration of the composition of the invention via inhalation has the effect of rapid dispersion to a large area of mucosal tissues, as well as absorption by the blood for circulation of the composition of the invention to other GM1 cells of the animal. The invention is further embodied by administering the composition ophthalmically. Administration of the composition of the invention ophthalmically has the aim of exposing the mucosa of the eyes and associated tissues to the composition.

In a preferred embodiment, the mode of administration is oral. Administration of the composition of the invention orally has the effect of exposing the mucosa of the gastrointestinal tract to the composition. A suitable dose delivers the composition to all parts of the gastrointestinal tract. The invention contemplates all means of oral delivery, whether in a liquid oral dosage, as a solid tablet, or in a capsule form.

Further preferred embodiments administer the composition of the invention rectally or vaginally. Administration of the composition of the invention rectally or vaginally has the effect of contacting a mucosal surface with the composition for release to proximal mucosal tissues. Distal mucosal tissues may also receive the composition of the invention by diffusion.

Another aspect of the invention is the composition functioning to deliver a polynucleotide to any GM1 animal cell. Where the binding protein component of the composition has an affinity for the GM1 receptor, the composition could be delivered to any cell displaying the GM1 receptor.

GM1 ganglioside is found in the plasma membrane of cells throughout the body. However, the concentration of GM1 in the plasma membrane of different cell types varies significantly. Only in cell types with a certain critical level of GM1 concentration does GM1 actually act as a receptor for binding CTB. Different binding proteins interact with GM1 or aggregates of GM1 in different ways. This fact allows a person practicing the invention to design a gene therapy composition capable of limited targeting of a therapeutic gene to mucosal cells, or more general targeting to other cells possessing a certain concentration of GM1 in their membranes. This mode of delivering therapeutic genes therefore allows a degree of precision in selecting target cells not previously available.

The present invention contemplates the use of any protein capable of specifically binding to or associating with GM1 cells. For purposes of the invention, a GM1 cell is a cell of a vertebrate animal having ganglioside GM1 in its plasma membrane. Many of the proteins already listed before as mucosa-binding proteins are either known or believed to bind mucosa via the GM1 receptor, and are therefore also suitable for use in this aspect of the invention. Likewise, all of the kinds of polynucleotides discussed above are also appropriate for and contemplated in this aspect of the invention.

The delivery of the composition of the invention is not limited to mucosal cells alone. Many other cells display GM1 in varying concentrations. For example, neural cells are known to have high concentrations of GM1. Other cells whose surfaces have lower concentrations of GM1 may be primed for delivery of the composition of the invention by preincubation with GM1 followed by a stabilization period, then finally followed by dosage of the composition of the invention. Cells with different membrane components will have varying degrees of affinity for and rates of uptake of GM1. Accordingly, the invention can be practiced in such a way as to regulate the time and other conditions of incubation, together with the concentration of the exogenous GM1 and the time of stabilization, to modulate the effectiveness of the uptake of GM1 by the target cells and thereby modulate the ultimate dosage of the composition delivered. Thus, any cell type can potentially be selectively pretreated to enhance the efficiency of delivery of the composition of the invention.

Likewise, anti-GM1 antibodies can be used to block GM1 receptors and attenuate the affinity of high-GM1 membranes for the composition of the invention. The invention anticipates the use of both forms of pretreatment: either with antibodies against GM1 or with GM1 itself, either singly or in combination, to achieve the desired result.

As a nonlimiting example of the use of both forms of pretreatment, a tissue consisting of two major subpopulations of cells is selected for treatment with the low-GM1 cells being the most preferred target for delivery of the composition of the invention. First the entire tissue is treated with a solution containing anti-GM1 IgGs at an appropriate concentration. The IgGs occupy most or nearly all of the accessible GM1 in the membranes of the high-GM1 cells. After lavage to remove the excess antibody, GM1 is added to the tissue. Following the diffusion gradient, GM1 will insert into the low-GM1 membranes more than into the high GM-1 membranes, and the GM1 thus diffused will be largely free from antibody blocking. After a second lavage to remove non-inserted GM1, the tissue is ready for dosage of the composition of the invention, and specific delivery of the active polynucleotide is effected to the cells that were originally lowest in GM1.

The gene therapy aspect of the invention discloses a method for gene therapy in an animal by administering to an animal in combination a GM1-binding protein and a functional polynucleotide and then expressing the polynucleotide in cells of the animal. By this method, the function of the polynucleotide confers on the animal a therapeutic effect.

In a preferred embodiment of this aspect of the invention, the cells of the animal transiently express the delivered functional polynucleotide. Transient expression is characterized by an onset of expression eventually followed by a cessation of expression. The duration of expression is a function of the stability of the active

polynucleotide in the cell. Factors affecting the stability of the polynucleotide include: the nature and structure of the polynucleotide, the subcellular location of the polynucleotide, the availability of stabilizing proteins or lytic enzymes in the local area of the polynucleotide, and other physical parameters of the cell such as temperature, pH, and concentration of certain ions.

For many applications, transient expression is preferred because the product of the delivered gene or the activity of the polynucleotide would be deleterious in the long term. Therefore, in such cases, selection of a polynucleotide more likely to be transiently, and not stably, expressed, will be appropriate. The characteristics of polynucleotides that render them more or less stable in a host cell are known in the art, and a person of ordinary skill could select an appropriate polynucleotide for the particular practice of the invention.

An alternative preferred embodiment exists when the cells of the animal are stably transformed by the functional polynucleotide. Stable transformation is characterized by the actual integration of a polynucleotide into the genome of the host cell, and subsequent expression of the genes or other activity of the polynucleotide throughout the life of the cell.

Certain uses of the present invention will necessitate or benefit from stable transformation of the cells by the polynucleotide. For example, where the therapy involved is the replacement of a missing or nonfunctional gene, stable transformation of the cell would potentially cure the defect, where transient expression of the polynucleotide would necessitate continuing treatments.

The characteristics of polynucleotides that may alone or in combination make the polynucleotide more likely to stably integrate into the genome of the host cell are known in the art. A skilled artisan could select the desired characteristics for the polynucleotide to be delivered so as to maximize the likelihood of stable integration and transformation of the host cell.

EXAMPLE 1. Forming the Composition

Cholera toxin B subunit protein (CTB) is coupled to poly-L-lysine as described previously. Jung,G., Kohnlein, W., and Fiders, G. (1981) Biochem. Biophys. Res. Commun. 101:599-606; incorporated herein by reference. The CTB protein is reacted with a 7-fold molar excess of poly-L-lysine at pH 7.4 using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide present at 154 molar excess over poly-L-lysine. After 24 hours, the conjugate product is purified by chromatography and titrated with plasmid DNA using a gel retardation assay as described by Wu. Wu, G.Y., and Wu, C.H. (1987) J. Biol. Chem. 262:4429-4432; incorporated herein by reference. The DNA:protein complex is then used to form a composition appropriate for the preferred mode of delivery.

EXAMPLE 2. Modulated Immunity: Enhancement

A DNA sequence containing a strong promoter and a coding region encoding epitopes of the A8 and VDIV proteins of *Chlamydia trachomatis* is complexed with CTB by the polylysine method as discussed above. A small amount of Cholera toxin A (CTA) subunit is included in the composition as an adjuvant. See Czerkinsky, C., and Holmgren, J. (1995) The Immunologist 3/3:97-103; and Allen, J., Locksley, R.M., and Stephens, R.S. (1991) J.

Immunol. 147:674-679; both incorporated herein by reference. The composition is delivered once via vaginal suppository to *Chlamydia*-free volunteers. A *Chlamydia*-free control group is given a placebo suppository. At six one-week intervals, samples of serum and vaginal mucus are taken from individuals of both groups. ELISA assays on serum samples show the presence of IgG specific for A8/VDIV in the treatment group and not in the control group. Similar assays on mucus samples demonstrate that the treatment group has a high titer of sIgA specific for A8/VDIV while individuals of the control group show no such reaction. A subsequent historical study of both groups shows a lower incidence of *Chlamydia* infections among members of the treatment group compared with members of the control group.

EXAMPLE 3. Modulated Immunity: Reduced Inflammatory Response

A DNA sequence containing a strong promoter and a chimeric coding region encoding a fusion of both CTB and the heat shock protein (HSP 57) of *Chlamydia trachomatis* is complexed with CTB protein by the polylysine method as discussed above. The composition is delivered once via vaginal suppository to patients suffering from *Chlamydia* infections and pelvic inflammatory disease (PID). A control group of similarly infected patients is given a placebo suppository. At six one-week intervals scratch tests and tine tests are performed on the forearms of individuals of the two groups using whole *Chlamydia* lysates. Individuals of the two groups are also surveyed for determination of the severity of PID symptoms. The treatment group displays a markedly diminished sensitivity in the scratch and tine tests, as well as diminished symptoms of PID. The control group shows no such results.

EXAMPLE 4. Modulated Immunity: Simultaneous Enhancement and Reduced Inflammatory Response

A vaginal suppository containing both the DNA construct from Example 2 and the DNA construct from Example 3 is administered to volunteers infected with *Chlamydia*. A placebo suppository is administered to an otherwise similar control group. The individuals of the treatment group experience both decreased symptoms of PID and enhanced cell-mediated immunity to Chlamydia. Mucus samples show high titers of sIgA and IgG against A8/VDIV, but very little reaction to scratch and tine test using whole *Chlamydia* lysates. The collateral "bystander tissue" damage that is characteristic of PID is therefore diminished. There are no comparable results among members of the control group.

EXAMPLE 5. Toleration of Organ Transplants

A set of recombinant DNA plasmids is constructed, each containing DNA encoding one of various MHC-1 proteins under control of a mammalian promoter. The resulting plasmid bank is characterized and cataloged, and batches of each plasmid are complexed with CTB using the polylysine method as described above to create an organ tolerization treatment bank.

An organ donor is screened to determine her MHC-1 genotype. The results of the screen are matched with the appropriate tolerization composition from the bank discussed above.

A patient about to receive the donated organ is given doses of the tolerization composition as an atomized dispersion via inhalation, as a rectal suppository, and orally as an enteric-coated tablet. The patient develops a rapid "oral tolerance" to the foreign MHC-1 protein and displays a much-diminished nonself recognition of the donated organ. Because of the duration of expression and the abundant expression of the nonself MHC-1 gene in the mucosa-associated lymphoid tissues (MALT), the acquired tolerance is more long-lasting than would have been the case with any more conventional approach to oral tolerization using the MHC-1 protein itself, rather than the gene.

EXAMPLE 6. Treatment of an Autoimmune Disease

Patients suffering from an autoimmune form of diabetes are orally administered a composition consisting of a chimeric gene encoding both CTB and human insulin under control of a strong viral promoter, wherein the DNA of the chimeric gene was complexed with CTB protein by polylysine as described above. The chimeric gene is delivered to the intestinal mucosa where it is internalized by cells of the MALT. These cells express a chimeric fusion protein consisting of CTB and insulin, which in combination effect a tolerization of the mucosal and system immune system to the insulin epitopes. The patients' autoimmunity to insulin is diminished and the progress of their diabetes is slowed.

Other, nondiabetic, patients participating in a long-term study are also administered the composition. The subsequent onset of diabetes among the volunteers is lower than among individuals of an otherwise similar control group.

EXAMPLE 7. Gene Therapy

A composition is prepared consisting of LTB and non-mutant mRNA corresponding to the mutant gene that is causative of cystic fibrosis (CF). The composition is administered twice daily to CF patients both orally and via inhalation. The composition is localized to the mucosa of the lungs and the gastrointestinal tract where the enzyme for which the CF patients are deficient is efficiently and transiently produced. The symptoms of CF in the patients thus treated are much diminished.

WHAT IS CLAIMED IS:

1. A composition for mucosal delivery of a polynucleotide comprising:

a mucosa-binding protein having an affinity for determinants of vertebrate mucosal tissues; and

a polynucleotide in association with said binding protein.

2. The composition of Claim 1 wherein the mucosa-binding protein is selected from the group consisting of CT, CTB, LT, bacterial fimbriae proteins, animal lectins, plant lectins, and lectin-like proteins.

3. The composition of Claim 1 wherein the mucosa-binding protein is selected from the group consisting of cholera toxin and its derivatives.

4. The composition of Claim 1 wherein the mucosa-binding protein is an antibody.

5. The composition of Claim 4 wherein the antibody is directed against the GM1 receptor.

6. The composition of Claim 1 wherein the polynucleotide is DNA.

7. The composition of claim 6 wherein the DNA is of genomic origin.

8. The composition of Claim 6 wherein the DNA is cDNA.

9. The composition of Claim 6 wherein the DNA is recombinant.

10. The composition of Claim 6 wherein the DNA contains a promoter region and a coding region.

11. The composition of Claim 6 wherein the DNA encodes an antisense message.

12. The composition of Claim 1 wherein the polynucleotide is RNA.

13. The composition of Claim 12 wherein the RNA is mRNA.

14. The composition of Claim 12 wherein the RNA is antisense.

15. The composition of Claim 12 wherein the RNA is a ribozyme.

16. The composition of Claim 1 in the form of an atomized dispersion.

17. The composition of Claim 1 in the form of a suppository.

18. The composition of Claim 1 wherein the mucosa-binding protein and the polynucleotide exist in the form of a mixture.

19. The composition of Claim 1 wherein the mucosa-binding protein and the polynucleotide are chemically coupled.

20. The composition of Claim 1 wherein the mucosa-binding protein and the polynucleotide are encapsulated together.

21. A method for modulating immunity in an animal comprising:

administering to an animal in combination a mucosa-binding protein and a polynucleotide encoding an immunogen; and

expressing said polynucleotide in said animal whereby said animal generates an immune response to the product of said polynucleotide.

22. The method of Claim 21 wherein said modulation is an enhanced immune response.

23. The method of Claim 22 wherein said enhanced immune response is mucosal.

24. The method of Claim 22 wherein said enhanced immune response is systemic.

25. The method of Claim 22 wherein said enhanced immune response is for contraception.

26. The method of Claim 21 wherein said modulation is a diminished immune response.

27. The method of Claim 26 wherein said diminished immune response is mucosal.

28. The method of Claim 26 wherein said diminished immune response is systemic.

29. The method of Claim 26 wherein said diminished immune response is for desensitization to allergens.

30. The method of Claim 26 wherein said diminished immune response is for toleration of organ and tissue transplants.

31. The method of Claim 26 wherein said diminished immune response is for treatment of an autoimmune disease.

32. The method of Claim 21 for preexposure vaccination against a selected epitope.

33. The method of Claim 21 for preexposure desensitization to a selected epitope.

34. The method of Claim 21 for postexposure enhanced immune recognition of a selected epitope.

35. The method of Claim 21 for postexposure desensitization to a selected epitope.

36. The method of Claim 21 wherein the mode of administration is nasal.

37. The method of Claim 21 wherein the mode of administration is oral.

38. The method of Claim 21 wherein the mode of administration is rectal.

39. The method of Claim 21 wherein the mode of administration is vaginal.

40. The method of Claim 21 wherein the mode is administration is inhalation.

41. The method of Claim 21 wherein the mode of administration is ophthalmic.

42. A composition for delivery of a polynucleotide to a GM1 animal cell comprising:

a binding protein having an affinity for the GM1 receptor; and

a polynucleotide in association with said binding protein.

43. The composition of Claim 42 wherein the binding protein is selected from the group consisting of CT, CTB, LT, LTB, bacterial fimbriae proteins, animal lectins, plant lectins, and lectin-like proteins.

44. The composition of Claim 42 wherein the binding protein is selected from the group consisting of cholera toxin and its derivatives.

45. The composition of Claim 42 wherein the binding protein is an immunoglobulin.

46. A method for gene therapy in an animal comprising:

administering to an animal in combination a GM1-binding protein and a functional polynucleotide; and

expressing said polynucleotide in cells of said animal whereby the function of said polynucleotide confers on said animal a therapeutic effect.

47. The method of Claim 46 whereby said cells of said animal transiently express said functional polynucleotide.

48. The method of Claim 46 whereby said cells of said animal are stably transformed by said functional polynucleotide.